CLXXXVII. THE IDENTIFICATION OF LINOLEIC AND LINOLENIC ACIDS.

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THE characterisation of linoleic and linolenic acids by oxidation respectively to tetra- and hexa-hydroxystearic acids is at present less satisfactory than that of oleic acid as dihydroxystearic acid. We have recently examined a number of reactions of these acids and their esters in the hope of finding more conclusive means of identifying them in natural fats. The results obtained afford a more quantitative comparison of different oxidation processes and products than was available hitherto, but we have not succeeded in obtaining good yields of crystalline derivatives, suitable for precise quantitative recognition of either the di- or the tri-ethenoid acid.

It should be emphasised at the outset that linoleic and linolenic acids can both only be obtained in a relatively pure condition by separating them in the form of crystalline tetra- or hexa-bromo-addition compounds and then de-brominating the latter. Further, when either of the natural acids is treated with bromine, less than half is obtained as an insoluble crystalline bromo-addition product; moreover the acids regenerated from the latter, when again treated with bromine, again give less than 50 % yields of the crystalline bromo-addition products[1]. Thus Rollett [1909] showed that debromination of the crystalline tetrabromostearic acid (M.P. 114°, insoluble in light petroleum) from linoleic acid gave an "α"-linoleic acid which, on being treated in light petroleum with bromine, yielded the insoluble tetrabromostearic acid in an amount corresponding to not more than 43 % of the "α"-linoleic acid; the remaining product was a soluble tetrabromostearic acid, liquid at the ordinary temperature. Lino-lenic acid was found to exhibit similar behaviour, the hexabromostearic acids produced by addition of bromine differing in this case in their solubilities in ethyl ether.

Tetra- or hexa-hydroxystearic acids have usually been respectively obtained from linoleic or linolenic acids by the action of cold aqueous solutions of potassium permanganate. Hazura [1887] oxidised the "liquid" (unsaturated) acids of sunflower oil in this manner and obtained two tetrahydroxystearic acids, M.P. respectively 160–162° and 173–175°, in amounts apparently corresponding with about 24 and 7 % of the theoretical yield. Rollett [1909], employing regenerated "α"-linoleic acid, similarly obtained about 33 % of the theoretical yield of crude tetrahydroxystearic acids, apparently mainly the form of M.P. 173°. Nicolet and Cox [1922] record a yield of 54 % of the weight of "α"-linoleic acid taken (*i.e.* 43 % of theory) in the form of a mixture of tetrahydroxystearic acids, which melted at 162–168° and was resolved, after a long process of crystallisa-tion from water (containing a trace of hydrochloric acid) into the two acids,

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[1] The acids regenerated from the insoluble bromostearic acids have usually been termed "α"-linoleic or "α"-linolenic acids, whilst those from the soluble bromo-addition products are referred to as "β"-acids. This nomenclature, which has no configurational meaning, will be retained for convenience in the present paper.

M.P. 153° and 170°. Other workers have described tetrahydroxystearic acids melting over the range 152–177°; the two isomeric tetrahydroxystearic acids produced by alkaline permanganate oxidation of "α"-linoleic acid are generally accepted to be: (a) an acid ("α-sativic acid"), M.P. 154–157°, moderately soluble in hot and sparingly in cold water, crystallisable from alcohol or ethyl acetate, and (b) an acid ("β-sativic acid"), M.P. 171–173°, slightly soluble in hot water, very sparingly soluble in hot ethyl acetate and crystallisable from acetic acid or from a somewhat large volume of ethyl acetate.

Nicolet and Cox [1922] also observed that, on addition of hypochlorous or hypobromous acid to "α"-linoleic acid, dichloro-(bromo-)dihydroxystearic acids were produced smoothly and could be converted into (impure) oily tetra-acetoxy-stearic acids (85 % yield). The latter, on hydrolysis, gave small amounts of two other isomeric crystalline tetrahydroxystearic acids (yield only 14 %), which melted at 144·5° and 135°; the acids of M.P. 153° and 170° were not obtained by this procedure. The acid of M.P. 144° was also obtained by Smith and Chibnall [1932] when they oxidised linoleic acid with hydrogen peroxide and acetic acid.

In the case of the triethenoid linolenic acid, Hazura [1887; 1888] oxidised the acids from linseed oil with alkaline permanganate and obtained two hexa-hydroxystearic ("*iso*linusic" and "linusic") acids: (a) M.P. 173–175° (yield 12–16 % of the mixed fatty acids) and (b) M.P. 203° (yield 3–4 % of the mixed fatty acids), together with the two tetrahydroxystearic acids from linoleic acid; he also records the production of the acid, M.P. 201°, from regenerated "α"-linolenic acid. Rollett [1909] similarly obtained both hexahydroxystearic acids from "α"-linolenic acid, the yield of that melting at 203° being about 6 % of the theoretical amount.

Bauer and Kutsche [1925] state that ethyl linolenate does not react with hydrogen peroxide, but that it yields an oil, $C_{20}H_{34}O_5$, upon treatment with perbenzoic acid, which resembles that obtained on combination with gaseous oxygen and which contains 3 added oxygen atoms per mol. of linolenic acid.

EXPERIMENTAL.

In the course of the present work we have submitted linoleic and linolenic acids to oxidation with alkaline permanganate solutions, with hydrogen peroxide and acetic acid, and with perbenzoic acid, and have also examined the action of Prévost's reagent (silver benzoate and iodine). An attempt (unsuccessful) was also made to convert linoleic acid into a diacetylenic derivative, corresponding with the production of stearolic from oleic acid. Safflower seed oil, which contains a high proportion of linoleic acid unaccompanied by linolenic acid, and of which we had a convenient supply, was used as the chief source of linoleic acid, whilst linolenic acid was obtained from linseed oil. The regenerated "α"-forms of the two acids were employed in many cases, but we supplemented these experiments by others upon the mixed acids from the respective oils. In the case of linoleic acid, we also examined the behaviour of the regenerated "β"-acid obtained from the petroleum-soluble bromination products and that of "α"-linoleic acid which had been submitted to isomerisation by oxides of nitrogen. Comparison of the results from the oxidation of the "α"-acids with those from the oxidation of the mixed fatty acids without bromination has led us to consider that the linoleic and/or linolenic acid of the original seed-fats very probably each exists in only one form, and that mixtures of hydroxy- or bromo-derivatives are produced in consequence of isomeric changes which accompany oxidation or bromination.

LINOLEIC ACID.

"α"-*Linoleic acid*. Methyl "α"-linoleate (I.V. 164·3) was prepared by de-bromination (with zinc and methyl alcoholic hydrogen chloride) of the tetra-bromostearic acid (M.P. 114°) obtained by addition of the theoretical amount of bromine to a solution of the mixed fatty acids of safflower seed oil in light petroleum (B.P. 80–100°) at 0°. "α"-Linoleic acid was subsequently prepared as required from the distilled methyl ester.

"β"-*Linoleic acid*. "α"-Linoleic acid was brominated at 0° in light petroleum (B.P. 40–60°); after filtration of the crystalline tetrabromostearic acid, the solution was washed with sodium thiosulphate solution and the petroleum then removed. The residual oil (Br found: 50·5 %; $C_{18}H_{32}O_2Br_4$ requires 53·3 %) was debrominated as above and finally yielded "β"-linoleic acid (I.V. 133·1, Br 2·1 %). It is to be noted that, although the acid contained but little bromine, the I.V. was very low[1] (linoleic acid, I.V. 181·4); this may well indicate that some of the petroleum-soluble products of bromination have undergone some further change during debromination. (In the debromination of the crystalline bromo-acid, it has been definitely shown [Rollett, 1909; Hilditch and Vidyarthi, 1929] that the ethenoid bonds reappear in their original positions.)

Oxidation with alkaline permanganate solutions.

We have employed the conditions of oxidation given by Lapworth and Mottram [1925] in the case of oleic acid, as well as those described by Hazura [1887] for linoleic and linolenic acids. The first-named authors advise the use of extremely dilute solutions, which minimises further oxidation of the hydroxy-stearic acids. This advantage is somewhat discounted, in the cases of linoleic and linolenic acids, by the solubility in water of some of the oxidation products and we found difficulty in recovering all the tetrahydroxystearic acids (especially that of M.P. 155°) from the large volume of water present after oxidations by the Lapworth and Mottram method.

(i) "α"-*Linoleic acid (modified Hazura process)*. Potassium permanganate (5 g.) in ice-cold water (200 ml.) was added to a solution of the acid (4·8 g.) and potassium hydroxide (2·8 g.) in water (300 ml.) at 0°. After standing at 0° for 24 hours, the solution was decolorised with SO_2 and made acid with concentrated HCl (150 ml.). The solid tetrahydroxystearic acids were filtered off and separated by fractional crystallisation from ethyl acetate (M.P. 173°, 1·3 g.; M.P. 155°, 2·6 g.). The aqueous filtrates were evaporated to dryness and extracted with hot alcohol, which removed a certain amount (1·1 g.) of viscous material from which however no further crystalline products could be extracted.

(ii) "α"-*Linoleic acid (Lapworth and Mottram process)*. Potassium perman-ganate (8 g.) in ice-cold water (400 ml.) was added to a solution of the acid (4·8 g.) and sodium hydroxide (4·8 g.) in water (2·5 litres) at 0°. After standing for 5–10 minutes, the solution was decolorised, concentrated HCl (150 ml.) added, and the tetrahydroxystearic acids filtered and separated as above (M.P. 173°, 0·9 g.; M.P. 155°, 1·4 g.; from the ethyl acetate mother-liquors 0·4 g. of material which melted indefinitely at about 128° was recovered. This appeared to consist of the acid, M.P. 155°, admixed with some azelaic or suberic acid).

(iii) *Mixed fatty acids of safflower oil (modified Hazura process)*. The mixed acids include about 60 % of linoleic and about 25 % of oleic acid [*cf.* Jamieson and Gertler, 1929]. The mixed acids (4·8 g.) were dissolved with potassium hydroxide (2·8 g.) in water (300 ml.) and mixed at 0° with a solution of potassium

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[1] Rollett [1909] records I.V. 159·5 for "β"-linoleic acid.

permanganate (3·7 g.) in water (200 ml.). After standing at 0° for 24 hours, the products were isolated as in (i), and were found to consist of:

> (a) 0·67 g. tetrahydroxystearic acid, M.P. 172°.
> (b) 0·75 g. tetrahydroxystearic acid, M.P. 151–153°.
> (c) 0·24 g. M.P. 130–135° (impure dihydroxystearic acid).

The yields of the tetrahydroxystearic acids M.P. 172° and 151–153° correspond respectively with about 18·5 and 20 % of the theoretical, calculated on 60 % of linoleic acid in the mixed safflower fatty acids. The reduced yield of dihydroxystearic acid from oleic acid, when a mixture of oleic and linoleic acids is oxidised by aqueous alkaline permanganate, was noted by Lapworth and Mottram [1925]. It is important to bear in mind this mutual influence of one unsaturated acid upon another, if the alkaline permanganate oxidation procedure is to be employed as an analytical method for the characterisation of oleic, linoleic or linolenic acid.

(iv) Freshly-distilled methyl "α"-linoleate was shaken with mercury (0·2 g.) and nitric acid (sp. gr. 1·4, 0·5 ml.) in a stoppered bottle at room temperature for one hour in order to effect geometrical isomerisation of the ethenoid linkages [Griffiths and Hilditch, 1932]. The product was washed free from nitric acid and hydrolysed. The acids obtained (4·8 g., I.V. 137·5) were oxidised by the Lapworth and Mottram procedure, when there were finally obtained 0·35 g. (M.P. 173°) and 0·6 g. (M.P. 155°).

(v) "β"-Linoleic acid (3·0 g.) was oxidised by the modified Hazura procedure, but only 0·10 and 0·07 g. of the crystalline acids M.P. respectively 173° and 155° were recovered. The main product (2·7 g.) was recovered by extraction with ether of the aqueous acid oxidation products as a mixture of oily acids (I.V. 13·7, mean equiv. wt. 76). On long standing in a vacuum desiccator the oil deposited a trace of crystalline acid which appeared to be impure suberic acid. The low equivalent of the main product indicates of course that a considerable amount of the "β"-linoleic acid had undergone disruptive oxidation into short-chain acids.

The main results of the above experiments may be given in tabular form: in each case 4·8 g. of linoleic acid were oxidised and the weights and yields (% of theory) of the acids, M.P. 155° and 173°, which were obtained are shown.

		Tetrahydroxystearic acids			
		M.P. 155°		M.P. 173°	
Acid oxidised	Conditions of oxidation	g.	Yield %	g.	Yield %
Mixed safflower acids (ca. 60 % linoleic)	Hazura	0·75	20	0·7	18
"α"-Linoleic	Hazura	2·6	43	1·3	22
"α"-Linoleic	Lapworth and Mottram	1·4	23	0·9	15
"α"-Linoleic, after "elaidinisation" with oxides of nitrogen	Lapworth and Mottram	0·6	10	0·35	6
"β"-Linoleic	Hazura	0·1	2	0·15	3

The yields of the two tetrahydroxystearic acids in our experiment with "α"-linoleic acid by the Hazura method (in all 65 % of theory) are higher than previously recorded and are not, if readily reproducible, unsatisfactory as a method of identification. In the other two cases quoted, we obtained combined

yields for the two acids of 38 % of theory, a figure intermediate between those of Nicolet and Cox (43 %), Rollett (33 %) and Hazura (31 %). We believe that much of the observed variation in yield is to be ascribed to the difficult recovery and separation of the products of the oxidation. In particular, we feel that the quantitative recovery of the acid of M.P. 155°, because of its appreciable solubility in cold water and of the relatively large volumes of water necessarily present, is bound to be uncertain. In some of our experiments especial attention was paid to this feature and the aqueous solutions were considerably reduced in bulk by evaporation, a procedure which adds greatly to the length of time consumed in the operations but which probably accounts for the high yield of this acid obtained in the instance recorded.

The most significant point brought out in the table above is that the linoleic acid present in the mixed acids of safflower oil appears to have given yields of the two tetrahydroxystearic acids of much the same order as those usually observed in the case of "α"-linoleic acid. In conjunction with the evidence obtained in the oxidation of "β"-linoleic acid (cf. below), this seems fairly conclusive evidence that the natural linoleic acid in the seed fat behaves, so far as the alkaline permanganate oxidation is concerned, as if it were the "α"-acid.

The small yields of the acids, M.P. 155° and 173°, from the oxidation of the "α"-linoleic acid after its treatment with the "elaidin" reagent show, if indeed proof be required, that the proportions of these acids produced are dependent on the geometrically isomeric forms of $\Delta^{9:10, \, 12:13}$-octadecadienoic acid which may be present. In this experiment, in addition to the acids shown in the table, a very small amount (0·1 g.) of a third tetrahydroxystearic acid, M.P. 140–141° (the melting point of which was depressed on admixture with the acid of M.P. 146°, v. infra) was also produced.

. The oxidation of "β"-linoleic acid with alkaline permanganate (which, curiously, does not seem to have been investigated by the earlier workers) gave mainly an oil (I.V. 13·7, mean equiv. wt. 76) from which a very small amount of an acid (apparently suberic acid) was isolated. The general properties of this substance indicate that the "β"-acid had undergone profound disruptive oxidation under the conditions of the Hazura process. "β"-Linoleic acid yields extremely small proportions of the two tetrahydroxystearic acids, M.P. 155° and 172°, less in fact than the "elaidinised" or isomerised "α"-acid. If therefore natural linoleic acid (e.g. in seed fats) were indeed a mixture of isomeric "α"- and "β"-forms, the yields of these two acids would be quite uncertain criteria of the presence of the acid. We feel however that all the properties of the "β"-acid suggest that it may be a somewhat complex mixture of several transposition products resulting from the action of bromine on the natural acid, and that the latter, although it gives rise (like the "α"-acid) to two forms of tetrahydroxystearic acid, may well be a stereochemical individual, probably, in view of the independent work of Nicolet and Cox [1922] and of Suzuki et al. [1931; 1932], the cis-cis-$\Delta^{9:10, \, 12:13}$-octadecadienoic acid. This view is certainly reinforced by the similarity in yields of the two tetrahydroxystearic acids, M.P. 155° and 172°, from natural and from "α"-linoleic acid.

Oxidation with hydrogen peroxide and acetic acid.

(i) Methyl "α"-linoleate (10 g.) was dissolved in glacial acetic acid (100 ml.); perhydrol (35 % H_2O_2, 10 ml.) was added at 0° and the solution kept at this temperature for 2 days, during which 30 ml. more of perhydrol were added (with 100 ml. of acetic acid to maintain complete solution of the ester). The solution was kept at 0° for a further 7 days. It was then poured into excess

of 7 % aqueous ammonia solution and the oxidation products were removed by extraction with ether and converted into the free acids as described by Hilditch and Lea [1928]. The ether solution of the free acids deposited a solid compound (1·8 g.) which, after recrystallisation from ethyl acetate, melted at 146°. (Found: C, 61·6; H, 9·9 %. $C_{18}H_{36}O_6$ requires C, 62·1; H, 10·3 %.) This tetra-hydroxystearic acid was distinct from that melting at 155° (a mixture of the two acids melted indefinitely between 141° and 148°).

The residue (7·4 g.) from the ether solution was an oil (I.V. 6·9) which, after standing for some weeks, deposited a small quantity (0·3 g.) of a solid acid which melted, after crystallisation from ethyl acetate, at 126°. (Found: C, 62·7; H, 10·3 %.) No further crystalline products were obtained from this oil, which formed the greater part of the products of oxidation.

The yields of the two isomeric tetrahydroxystearic acids, M.P. 146° and 126°, were respectively about 14 and 2 % of the theoretical.

(ii) Methyl "α"-linoleate (10 g.) was similarly oxidised in acetic acid solution (100 ml.) with perhydrol (40 ml., added in two portions) at 70–80° for 10 hours. The product was worked up as in (i), and a small quantity (0·4 g.) of the acid, M.P. 146°, separated from the ether solution of the recovered acids. The remainder (5·0 g.) of the product was an oil (I.V. 14·1) from which boiling water removed a very small quantity of a solid acid; this, on crystallisation from benzene, melted at 95° and appeared to be azelaic acid; no other crystalline acids could be obtained from the oil. The loss of material (nearly 5 g.) in this experiment is noteworthy, and suggests considerable formation of water-soluble acids of low molecular weight; it was also observed that the alcoholic alkaline solution became very dark in colour during the hydrolysis of the oxidised esters.

(iii) The methyl esters (10 g.) of the mixed fatty acids from safflower seed oil were treated in glacial acetic acid (150 ml.) with perhydrol (30 ml.) at 0° for a week under the conditions described in the preceding experiment (i). The products finally isolated included:

0·37 g. tetrahydroxystearic acid, M.P. 146° (equivalent to about 5 % of the linoleic acid present; M.P. unchanged on admixture with the corresponding acid, M.P. 146°, from experiment (i));

0·1 g. tetrahydroxystearic acid, M.P. 119–121°, which was apparently an impure specimen of that obtained in experiment (i) (mixed M.P. 121–125°). This corresponds with about 1 % of the linoleic acid present;

1·1 g. 9:10-dihydroxystearic acid, M.P. 92° (M.P. 93° when mixed with pure 9:10-dihydroxystearic acid of M.P. 95°);

5·9 g. of viscid, indefinite products of oxidation (I.V. 16·3).

(iv) "β"-Linoleic acid (3·9 g., I.V. 133) was similarly oxidised in glacial acetic acid (60 ml.) with perhydrol (12 ml.) at 0° for a week. Almost the whole of the products recovered was in the form of a very viscous, gum-like material which showed the typical reactions of an organic peroxide (4·0 g., I.V. 11·9. On heating under vacuum at 100° for 1 hour, evolution of gas took place and the I.V. of the substance increased to 14·3). A very small amount (0·03 g.) of tetra-hydroxystearic acid, M.P. 141–144°, was the only other product isolated.

It seems certain that the tetrahydroxystearic acid, M.P. 146° (which was similarly obtained from linoleic acid by Smith and Chibnall [1932]) is the same compound as that described by Nicolet and Cox [1922]; but the other isomeric acid, M.P. 126°, does not appear to be the same as the acid of M.P. 135° obtained by the latter authors.

Towards hydrogen peroxide (or peracetic acid), as well as towards alkaline permanganate therefore the behaviour of the natural linoleic acid differs ma-

terially from that of either oleic or elaidic acid. The monoethenoid acids (especially elaidic) are readily converted by hydrogen peroxide and acetic acid into one or other of the isomeric 9:10-dihydroxystearic acids; moreover, in the case of oleic acid the dihydroxy-acid produced is that melting at 95°, whilst elaidic acid yields the other form, M.P. 132° (*i.e.* the opposite acid is produced in each case to that obtained by the use of alkaline permanganate). With linoleic acid, two new tetrahydroxystearic acids are produced, in very small yields, but neither has apparently any simple relationship to those obtained in the alkaline permanganate oxidation. This is illustrated by the fact that alkaline permanganate oxidation of methyl "α"-linoleate which had been "elaidinised" did not furnish either of the acids, M.P. 146° or 126°. (The possible stereochemical relationships of isomeric 9:10:12:13-tetrahydroxystearic acids have been discussed by Nicolet and Cox [1922].)

Linoleic acid and esters are largely converted at 0° by hydrogen peroxide into viscous products of an organic peroxide or "linoxyn"-like character, a behaviour which is exhibited to a considerably smaller extent by monoethenoid acids or esters [Hilditch and Lea, 1928]; at higher temperatures (70°) breakdown of the carbon chain evidently becomes an important feature of the process. As a practical method for characterisation of linoleic acid, the hydrogen peroxide-acetic acid oxidation is seen to be definitely inferior to oxidation with alkaline permanganate.

Oxidation with perbenzoic acid.

Perbenzoic acid (9·5 g., prepared by the method of Hibbert and Burt [1925] in 190 ml. chloroform) was left with methyl "α"-linoleate at 0° in the dark for 3 days. From the cold alcoholic alkaline solution employed to hydrolyse the resulting esters [*cf.* Böeseken, 1926; Steger and van Loon, 1927], there separated a crystalline potassium salt, the acid (2·6 g.) from which melted, after crystallisation from 70 % alcohol, at 79°. (Found: C, 69·0; H, 10·8 %. $C_{18}H_{32}O_4$ requires C, 69·2; H, 10·3 %.) This corresponds with a dioxidostearic acid, but the compound could not be converted into a crystalline tetrahydroxystearic acid by either of the methods by which the above investigators have effected the transformation of mono-oxidostearic acids into 9:10-dihydroxystearic acids. The main product of the reaction was an oil (5·4 g., obtained from the products of cold alkaline hydrolysis of the esters after separation of the above-mentioned crystalline potassium salt). This deposited some solid on keeping, but attempts to separate and purify the latter were unsuccessful. The oil, originally mobile and pale in colour, darkened and became viscous on heating at 100° in a vacuum.

The yield of the dioxidostearic acid (about 25 % of the theoretical) was moderately satisfactory, at all events by comparison with those from most of the other processes discussed in the present paper.

Oxidation by means of silver benzoate and iodine in benzene.

The use of this reagent for the conversion of aliphatic ethenoid groups into diglycols has recently been proposed by Prévost [1933]. Since it does not seem to have been applied hitherto to the higher unsaturated fatty acids, we examined its action on the monoethenoid compounds, methyl oleate and elaidic acid, before employing it in the case of linoleic acid.

Methyl oleate. Dry silver benzoate (15·5 g.) was added to a solution of methyl oleate (10 g.) in benzene (200 ml.). To the boiling solution there was added a solution of iodine (8·7 g.) in benzene (50 ml.). After refluxing for 3 hours, silver iodide was removed by filtration, and the residue (13 g., I.V. 5·5), after removal

of the benzene, was hydrolysed by $N/2$ alcoholic KOH (250 ml.). The ether solution of the acids so obtained deposited a solid acid (4·1 g.), which, after recrystallisation from ethyl acetate, melted at 130° (unchanged on admixture with 9:10-dihydroxystearic acid, M.P. 131°). The acids which remained in solution in the ether were freed from benzoic acid and were mainly liquid. 1·2 g. of a solid acid were separated from them however which, on purification, melted at 95° (no depression in M.P. when mixed with 9:10-dihydroxystearic acid, M.P. 95°); the remainder of the material (3·8 g.) remained in the form of an oil.

Elaidic acid (10 g.) were treated in a manner similar to the above and finally yielded about 5·8 g. of a solid acid, which, on crystallisation, was found to consist entirely of the 9:10-dihydroxystearic acid of M.P. 95° (M.P. observed, 93–94°, unchanged on admixture with the pure acid). The isomeric dihydroxystearic acid (M.P. 131°) was not detected; the remainder of the product was an oil (4·0 g.).

It thus appears that, with the simple monoethylenic acids, Prévost's reagent leads to the production in about 50 % yield of a dihydroxystearic acid, the remainder being an oil of indefinite character; for the most part, also, the dihydroxystearic acid produced is the same form as that obtained by oxidation of the original ethylenic acid with alkaline permanganate.

When methyl "α"-linoleate was submitted to this process, however, it was almost wholly converted into a gummy material, from which extremely small amounts of the two tetrahydroxystearic acids, which result from the alkaline permanganate oxidation, were recovered. From 10 g. of methyl "α"-linoleate there was obtained a dark-coloured gum (11 g., I.V. 46·7), together with 0·3 g. tetrahydroxystearic acid, M.P. 153° and 0·1 g. (M.P. 168°) of an impure specimen of the isomeric acid of M.P. 173°.

Action of alcoholic alkali upon tetrabromostearic acid.

We re-examined the action (which has been studied by other workers) of ethyl alcoholic potash at different concentrations and temperatures upon the tetrabromostearic acid, M.P. 114°, derived from linoleic acid, but in no case, nor by the use of silver oxide, were we able to obtain any tetrahydroxystearic acids. Removal of hydrogen bromide, with the production of ethylenic acids which usually also retain one or more bromine atoms, appeared to be the predominant action in all cases [cf. Albitski, 1899; Suzuki and Maruyama, 1931; 1932].

We also heated tetrabromostearic acid with concentrated amyl alcoholic KOH in the hope of producing a diacetylenic analogue of stearolic acid (from dibromostearic acid, cf. Overbeck [1866]). Tetrabromostearic acid (M.P. 114°, 20 g.) was refluxed with a solution of sodium hydroxide (14·4 g.) in amyl alcohol (60 ml.) for 4 hours. After removal of the amyl alcohol by distillation in steam, 8·9 g. of oily acids (I.V. 135·0) were recovered. The lithium salts of these acids were completely soluble in 95 % acetone, whilst over 90 % of their lead salts were soluble in 95 % alcohol. It seems therefore that little or no diacetylenic acid had been produced, and that regeneration of octadecadienoic acids had mainly taken place.

LINOLENIC ACID.

Oxidations with alkaline permanganate solutions.

By oxidation of either "α"-linolenic acid or the mixed acids from linseed oil with alkaline permanganate we obtained the two hexahydroxystearic acids (M.P. 203° and 169°) in small yields of the order of those recorded by Hazura

[1888] and by Rollett [1909]. Attempts to increase the yields of these acids were uniformly unsuccessful.

(i) The aqueous soap solution (150 ml.) obtained from the hydrolysis of ethyl "α"-linolenate (4·7 g.) was mixed at 0° with a solution of potassium permanganate (12 g.) in water (400 ml.) and kept at 0° for a day. After decoloration and acidification with sulphuric acid a small amount of solid (0·2 g.) separated which, after crystallisation from alcohol, melted at 203°. (Found: C, 56·6; H, 9·8 %. $C_{18}H_{36}O_8$ requires C, 56·9; H, 9·5 %.) The aqueous filtrate was concentrated, after neutralising, to about 150 ml. and again made acid and filtered whilst hot, when 0·8 g. of acid, M.P. 180–188° (indef.) was separated; the filtrate, on cooling at 0° overnight, deposited 0·6 g. of acid, M.P. 174°. These two acid fractions were separately crystallised several times from alcohol, and eventually there were obtained in all (a) 0·3 g. more of the acid, M.P. 203°, and (b) 0·7 g. of an acid which finally melted at 166°. (Found: C, 57·2; H, 9·7 %.) Thus, in all, from 4·2 g. of linolenic acid there were isolated 0·5 g. of hexahydroxystearic acid, M.P. 203°, and 0·7 g. of hexahydroxystearic acid, M.P. 166°, representing together a yield of about 18 % of the theoretical.

(ii) The mixed fatty acids (50 g.) of linseed oil were dissolved in water (1 litre) with potassium hydroxide (12 g.) and mixed at 0° with a solution of potassium permanganate (50 g.) in water (2 litres) [Hazura and Friedreich, 1887]. After 24 hours, the solution was decolorised with SO_2 and made acid with dilute H_2SO_4, and the precipitated acids (which consisted mainly of hexahydroxystearic acid, M.P. 203° and the two tetrahydroxystearic acids, M.P. 155° and 173°) were filtered. These were boiled with water, when most of the hexahydroxystearic acid and of the tetrahydroxystearic acid, M.P. 155°, dissolved. The insoluble portion was recrystallised from alcohol and was found to be tetrahydroxystearic acid (0·2 g., M.P. 173°) containing a little hexahydroxystearic acid. (Found: C, 61·2; H, 10·1 %.)

When the water-soluble portion was treated with ethyl acetate, the hexahydroxystearic acid remained undissolved and was further purified by crystallisation from ethyl alcohol (1·1 g., M.P. 203°); the more soluble portion was twice recrystallised from ethyl acetate (0·1 g., M.P. 150°).

The original acid filtrates from the oxidation were neutralised and concentrated to 400 ml.; after again adding sulphuric acid, an acid separated which melted at 167°. This, the main yield of hexahydroxystearic acid, was mixed with small amounts of lower-melting hexahydroxystearic acids obtained in the above separations and yielded, after two crystallisations from methyl alcohol, a product (2·5 g.) which melted at 169–170°. (Found: C, 57·1; H, 9·3 %.)

Calculated on the amounts (approx. 40 % each) of linolenic and linoleic acids present in the original mixed linseed oil acids, the yields of the four products were about 5 % of hexahydroxy-acid, M.P. 203°, 10 % of hexahydroxy-acid, M.P. 169–170°, 0·8 % of tetrahydroxy-acid, M.P. 173°, and 0·5 % of tetrahydroxy-acid, M.P. 155°, as compared with theory. It is noteworthy that the yields of the tetrahydroxystearic acids in this experiment were much lower than when linoleic acid was oxidised under similar conditions, but in absence of linolenic acid. This behaviour has an exact parallel in the analogous falling-off in the yield of 9:10-dihydroxystearic acid from oleic acid when a mixture of the latter with more than a certain maximum of linoleic acids is oxidised with alkaline permanganate.

Oxidation with hydrogen peroxide and acetic acid.

Ethyl "α"-linolenate (10 g.) was dissolved in glacial acetic acid (200 ml.) and perhydrol (60 ml.) added, the solution being kept at 0° for 10 days. The material was then worked up as described in the case of the corresponding experiment with methyl "α"-linoleate, but no crystalline potassium salts separated, and the product obtained (6·5 g., i.v. 39·3) was a viscous oil from which no crystalline solids could be isolated. This oil showed the characteristic properties of an organic peroxide and, when heated at 100° in a vacuum, evolved gas abundantly, its i.v. increasing to 52·8. It thus closely resembled in properties the oils obtained as by-products during the similar oxidations of methyl oleate or elaidate [Hilditch and Lea, 1928]. Approximately one-third of the original ester had disappeared, presumably in the form of free acids removed during neutralisation of the acetic acid (solvent) with aqueous ammonia; so that a considerable proportion of the ester had undergone disruptive oxidation.

Oxidation with perbenzoic acid.

Ethyl "α"-linolenate (15 g.) in chloroform (305 ml.) was mixed with perbenzoic acid (21 g.) and the solution kept at 0° for 10 days. The products (isolated as in the case of the corresponding experiment with linoleic ester) were a pale yellow oil (3·7 g., i.v. 8·3) readily soluble in ether and a thick gum (10·6 g., i.v. 3·0, equiv. wt. 319·6) which was sparingly soluble in ether but readily so in alcohol. Like the corresponding material obtained under these conditions by Bauer and Kutsche [1925], the composition of this gum appears to approximate to that of a compound $C_{17}H_{29}(O_3)CO_2H$ (equiv. wt., calc., 326). No crystalline oxido-compounds were isolable, nor was it found possible to convert the gum into crystalline hydroxy-acids by hydrolysis with 5 % sulphuric acid dissolved in 50 % aqueous acetic acid (method of Böeseken [1926]).

SUMMARY AND DISCUSSION.

The above results may be summed up by saying that, so far as the characterisation of linoleic or linolenic acids is concerned, we have found no better method than the original process of oxidation with alkaline permanganate as proposed by Hazura, but that this procedure, at best, leads to the isolation of crystalline tetra- or hexa-hydroxystearic acids in yields which respectively correspond with only about 40 % of theory from linoleic, or 15–18 % of theory from linolenic, acid.

Of the alternative methods we have examined, the only promise was shown in the oxidation of linoleic acid by perbenzoic acid when, however, only a 25 % yield of a crystalline product resulted; with linolenic acid perbenzoic acid furnished no crystalline products. Hydrogen peroxide in acetic acid (peracetic acid) gave small yields of two other isomeric tetrahydroxystearic acids from linoleic acid, but no definite products in the case of linolenic acid. The other methods studied all led to negative results.

On the other hand, the data given in this paper (chiefly those which concern the oxidations with alkaline permanganate) lead to several definite conclusions of some importance. Firstly, we have already emphasised the similarity in yield of tetrahydroxystearic acids from the linoleic acid in a natural fat and from "α"-linoleic acid (prepared from the crystalline form of tetrabromostearic acid); taken in conjunction with the failure of "β"-linoleic acid to yield any

appreciable quantity of either of the crystalline tetrahydroxystearic acids (a feature apparently not previously examined), this points strongly to the likelihood that the linoleic acid of seed-fats is confined to one geometrical form. The latter evidently undergoes isomeric change or "inversion" to some extent during bromination or oxidation.

Secondly, our results leave us unsatisfied that the so-called "β"-linoleic acid is simply a mixture of other forms of $\Delta^{9:10, 12:13}$-octadecadienoic acid. From the oxidation results, it appears still to contain a certain amount (perhaps 10–12 %) of the "α"-acid; whilst, from its low i.v. and other considerations, we are inclined to suspect that part, at least, of this product has undergone more profound alteration than the mere conversion of *cis*- into *trans*-ethenoid linkages.

We consider the behaviour of "elaidinised" "α"-linoleic acid to be much more significant. Here we know that the action of oxides of nitrogen is confined to effecting *cis-trans* isomerisation (except for the subsidiary production of small proportions of stable nitroso- or nitro-addition products). The isomerised acid still furnished, as would be expected from an equilibrium mixture of isomerides, a certain diminished yield of the usual tetrahydroxystearic acids, but no others (except for a minute amount of the isomeride of M.P. 146°). We regard this as evidence that some at least of the remaining three geometrical isomerides of $\Delta^{9:10, 12:13}$-octadecadienoic acid do not yield any crystalline tetrahydroxystearic acids under the conditions of the Hazura oxidation. Inability to obtain these acids from an octadecadienoic acid therefore only proves the absence of the isomeride present in seed fats (or the "α"-linoleic acid), and does not exclude the presence of other forms of the $\Delta^{9:10, 12:13}$-acid (or, of course, of other positional isomerides).

Another point which has emerged during the present study is that the isomeric tetrahydroxystearic acids obtained from linoleic acid on the one hand with alkaline permanganate and on the other hand with hydrogen peroxide in acetic acid do not bear any simple relationship to each other, whereas in the corresponding monoethenoid series (oleic and elaidic acids) there is a very definite interrelationship in this respect.

Although much of the evidence discussed in this communication is negative in tendency, it is felt desirable to place it on record for the following reasons: (*a*) the whole subject seemed to merit re-examination on a systematic basis in one investigation, in view of the somewhat divergent statements in the literature; (*b*) it was desirable to standardise, if possible, the criteria for natural linoleic (and linolenic) acids; (*c*) so far as the authors are concerned, the work forms an essential preliminary to the further investigation of the unsaturated C_{18}-acids of butter fat to which we return in the following paper.

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